Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Remarkable enhancement of Fenton degradation at wide pH range promoted by thioglycolic acid

Gokulakrishnan Subramanian and Giridhar Madras*

Dept. of Chemical Engineering, Indian Institute of Science, Bangalore 560012, India.

E-mail: gokul@chemeng.iisc.ernet.in; Fax: +91 80 23600683; Tel: +91 80 22932321

Contents

1. **Chemicals and reagents:**

Iron(II) sulphate heptahydrate, Iron(III) sulphate hydrate, Magnesium nitrate hexahydrate, Ammonium metavanadate, Terephthalic acid, Potassium bromide, Hydrogen peroxide (30% w/v), Acetic acid glacial (100 %), Hydrochloric acid (32 %), tert-Butyl alcohol (99 %), Thioglycolic acid, 4-Chlorophenol (99%), Nitroblue tetrazolium dichloride (NBT) and Bisphenol A were analytical grade purchased from Merck. 1, 10 Phenanthroline was purchased from Sigma-Aldrich. Ciprofloaxcin hydrochloride (Analytical grade), Nutrient Agar, Luria-Bertani broth were purchased from HiMedia Pvt Ltd, India. Acetonitrile (HPLC grade), Methylene Blue, Calcium chloride, Humic acid, Sodium acetate trihydrate, Sodium chloride, Sodium sulfate, Hydroxylamine hydrochloride, Sodium hydrogen carbonate, Sodium hydroxide, were of analytical reagent grade purchased from S.D Fine Chemicals Ltd., India.

All the solutions, buffers and aqueous reagents were freshly prepared with deionized water (resistivity 18.21 MΩ.cm) from the Millipore system. Stock solutions of MB (2.5 mM), Fe(II) (4 mM in 0.2 N HCl), TGA (20 mM) and H_2O_2 (100 mM) were freshly prepared. The Fe(II)TGA catalyst solution (100 ml) was prepared by adding 1.25 ml of the Fe(II) stock solution and 0.5 ml of TGA stock solution to deionized water (80 ml). The pH was adjusted to desired value by adding 0.1 N HCl or 0.1 N NaOH and the final volume was made to 100 ml with D. I water. This solution had [Fe(II)] = 50 µM and [TGA] = 100 µM. The Fe(II): TGA ratio was 1:2. The concentration of MB (ε_{664} = 61961 ± 149 $M^{-1}cm^{-1}$)[1] and H_2O_2 (ε_{250} = 22.7 $M^{-1}cm^{-1}$)[2] were determined spectrophotometrically. Simulated Ground Water (S.G.W) water was prepared as per the composition proposed by Liu *et al.,* except for the modification that borate was not added and pH was adjusted to 6 using NaOH.[3] *Caution! Chemicals such as TGA, NaOH and HCl corrosive and therefore, must be handled with care.*

2. Experimental Procedure:

2.1 Degradation studies:

Methylene Blue was selected for studying degradation kinetics because it has a strong absorption at 664 nm (ε_{664} = 61961 ± 149 M^{-1} cm^{-1}) that is favorable in two ways: (i) easy spectrophotometric monitoring and, (ii) negligible spectral interference due to the presence other analytes in reaction mixture. It should be noted that Fe(II) and hydrogen peroxide (H_2O_2) shows no absorption at 664 nm. Reactions were carried out in a borosilicate reaction vessel (7 x 9.5 cm) in dark conditions at ambient temperature (293 ± 3 K) under constant magnetic stirring. Degradation of MB by Fe(II)/TGA/H_2O_2 system was carried out by adding 1.25 ml of the Fe(II) stock solution and 0.5 ml of TGA stock solution to deionized water (80 ml). The pH was adjusted to desired value by adding 0.1 N HCl or 0.1 N NaOH. To this Fe(II)TGA catalyst solution, 0.4 ml of MB

stock solution was added the volume was adjusted to 99 ml. The degradation was initiated by adding 1 ml of H_2O_2 stock solution. The concentration of the reagents in this reaction mixture (100 ml) is as follows: [Fe(II)] = 50 μM; [TGA] = 100 μM; [MB] = 50 μM and [H_2O_2] = 1 mM. For degradation of MB by typical Fenton system (Fe(II)/H_2O_2), (without TGA), similar protocol was followed without the addition of TGA. The use of buffering reagents were avoided in order to prevent scavenging of $^{\bullet}OH$ by buffering agents. 1 ml of sample was withdrawn at desired time interval and the absorption at 664 nm was recorded using Shimadzu UV-Visible spectrophotometer; version 1700. The degradation results are expressed as C/C_0 vs. Time plot, where C_0 is the initial concentration of the MB before treatment, and C is the concentration of MB at desired time interval. Pseudo first order decay rate constant (*k'*, min^{-1}) and half life ($t_{1/2}$, min) values for MB degradation were determined from the slope of linear plot obtained from the log of ratio of concentration at specific time (C) to initial concentration (C_0).

2.2 Determination of H_2O_2 and Fe(II):

Concentration of H_2O_2 and Fe(II) was determined spectrophotometrically by using meta vanadate[4] and *o*-phenanthroline complexometry[3b, 5], respectively. Tert-butyl alcohol (k_{OH^*} = 6 x 10^8 M^{-1} s^{-1})[6] was used as $^{\bullet}OH$ scavenger to distinguish which oxidant, $^{\bullet}OH$ or Ferryl species ($Fe(IV)O^{2+}$) is mainly responsible for the degradation.[7]

2.3 Determination of hydroxyl radical and superoxide anion radical:

Detection of $^{\bullet}OH$ in Fe(II)/TGA/H_2O_2 and Fe(II)/ H_2O_2 system using Terapthalic acid (TA)[3b, 8] as probe compound was carried out by a protocol similar to degradation of MB as mentioned above except for the modification that instead of MB, TA was added. The reaction was initiated by adding H_2O_2 and the fluorescence at 425 nm was recorded at desired time intervals using Gen 5 Version 2.08.13 Fluorescence plate reader, Biotek instruments.

Detection of superoxide anion radical was carried out using nitroblue tetrazolium dichloride (NBT) as probe compound.[9] This assay was carried out by a protocol similar to TA assay mentioned above except for the modification that instead of TA, NBT was added along with 5% t-BuOH to scavenge hydroxyl radicals. The degradation of NBT was followed spectrophotometrically by monitoring absorbance at 280 nm at desired intervals.[9]

2.4 High Performance Liquid Chromatography (HPLC):

The degradation of contaminants such as 4-chlorophenol (4-CP), Bisphenol A (BPA) and Ciprofloxacin (Cpf)), by Fe(II)/H_2O_2 system and Fe(II)/TGA/H_2O_2 system was carried out following a similar protocol as mentioned above for MB degradation (section

2.1). The degradation of 4-CP, BPA and Cpf was monitored using HPLC (Waters 515 pump, C-18 column, Waters 2487 UV detector). The analysis was performed in an isocratic mode with water/acetonitrile/formic acid (60/40/0.1 volume %) as mobile phase. The flow rate for 4-CP, BPA and Cpf were set at 2 ml min^{-1}, 1.5 ml min^{-1} and 1 ml min^{-1}, respectively, and the chromatograms were monitored at 270 nm. Under these conditions, the retention time of 4-CP, BPA and Cpf were 2.3 min, 3.8 min and 1.2 min, respectively.

2.5 Total Organic Carbon (TOC):

TOC was measured using a Shimadzu TOC-VCSN analyzer. Samples, 20 ml were withdrawn after desired time intervals and analyzed immediately. The results are expressed as $[TOC]/[TOC]_0$, where TOC is the total organic carbon present in sample after desired treatment time, and TOC_0 is the initial total organic carbon present in sample before treatment.

2.6 Characteristics of simulated ground water and real water samples:

The performance of $Fe(II)/H_2O_2$ and $Fe(II)/TGA/H_2O_2$ systems were evaluated system in simulated ground water (S.G.W)[3] (Ca^{2+} = 19.5 mg/L; Mg^{+} = 2.9 mg/L; Br^{-} = 0.1 mg/L; Na^{+} = 42 mg/L; Cl^{-} = 30.5 mg/L; SO_4^{2-} = 36.7 mg/L; NO_3^{-} = 3.5 mg/L; CO_3 = 1 mM; Humic acid = 1 ppm), and also in three different natural water samples such as Tap Water, Waste Water Treatment Plant (WWTP) Effluent and Lake Water.

Tap water is treated water supplied by Bangalore city Municipal Corporation.

Waste Water Treatment Plant (WWTP) Effluent was collected from the WWTP located inside the IISc campus, Bangalore.

Lake water was collected from Sankey Lake, Bangalore.

The water quality parameters of these water samples are tabulated below:

Table S.I 1. Water quality parameters of real water samples:

Parameters	Method	Tap Water	WWTP Effluent	Sankey Lake Sample
Chemical Oxygen Demand (C.O.D)	IS:3025(P-58)2006	4 ppm	84 ppm	76.8 ppm
Biological oxygen demand (BOD)	IS:3025(P-44)1993	Below Detection limit	29 ppm	26 ppm
Total Organic Carbon (TOC)	ISO 8245:1999(E)	0.7 ppm	18 ppm	20.5 ppm
pH	pH meter	6.2 ± 0.1	7.2 ± 0.2	7.6 ± 0.3
Sulphate, as SO4	IS:3025 & APHA	11.92 ppm	17.30 ppm	19.56 ppm
Nitrate (as NO3)	IS:3025 & APHA	4.30 ppm	24.58 ppm	14.53 ppm
Chloride (as Cl)	IS:3025 & APHA	64.11 ppm	85.48 ppm	66.05 ppm
Phosphate (as PO_4)	IS:3025 (P-31) 2009	2 ppm	2.55 ppm	2.02 ppm
Total Hardness (as CaCO3)	IS:3025 (P-21) 2009	147.39 ppm	211.13 ppm	167.13 ppm
Total Dissolved Solids (mg/L)	IS:3025(P-16)-1984	336 ppm	499 ppm	586 ppm
Total Suspended Solids	IS:3025 (P-17) 1984	Absent	Absent	39 ppm

2.7 Microbiological assays:

Bacterial strains, Gram negative bacteria *Escherichia coli* (NCIM 2345), and Gram positive bacteria, *Staphylococcus aureus* (NCIM 2127) were cultured in LB medium using an orbital shaker set at 100 rpm, 37 °C for 12 h. Cells (at log phase) were harvested by centrifugation at 4000 R.P.M for 15 min and washed twice with sterile D.I water. A bacterial stock solution ~10^8 colony forming units (CFU) mL^{-1} was prepared by suspending cell pellet in an appropriate volume of S.G.W. Disinfection of bacteria by Fe(II)/TGA/H_2O_2 and Fe(II)/TGA/H_2O_2 systems was carried out under sterile conditions in laminar air flow following the same procedure as mentioned above for degradation studies (section 2.1) except that, instead of organic contaminant, appropriate volume of bacterial stock solution was added to the reaction mixture. The initial cell concentration for bacterial inactivation studies was fixed at ~10^6 CFU ml^{-1}. Enumeration of bacterial

cells was carried out by spread plate technique (Standard Method 9215C)[10]. Cell concentration before and after treatment (at specific time intervals) was determined by counting colonies after serial dilution and spread plating 0.1 mL of sample on NB agar plates, followed by incubation for 12 hours at 37°C. The accuracy of this procedure is 2 CFU ml^{-1}.[10]

2.8 Electro-Spray Ionization Mass Spectrometry (ESI-MS) analysis:

The fate of TGA in Fe(II)/TGA/H_2O_2 system was studied using Electro-Spray Ionization Mass Spectrometry. 2.5 ml of the Fe(II) stock solution (4 mM) and 1 ml of TGA stock solution (20 mM) were mixed, and the volume was made to 9 ml with deionised water after adjusting the pH to 6 using 0.1 N NaOH. To this Fe(II)TGA solution, 1 ml of H_2O_2 stock solution (100 mM) was added to initiate the reaction. The concentration of the reagents in this reaction mixture (10 ml) is as follows: [Fe(II)] = 1 mM; [TGA] = 2 mM; and [H_2O_2] = 10 mM. After 2 hours of treatment, 20 µL of this sample was injected directly using Cole Parmer syringe pump with the flow rate at 4 µL/min. Dry gas was kept 5 L/min and nebulizer pressure was kept 10 psi. Sample was scanned in positive and negative ion modes using HCT Ultra PTM Discovery System (ETD II- Bruker Daltonics) mass spectrometer.

2.9 Evaluation of toxicity:

For evaluation of toxicity of Fe(II)/TGA/H_2O_2 treated water against bacteria, two concentrations of reagent dose (i) optimized concentration [*denoted as 1x*; [Fe(II)] = 50 µM; [TGA] = 100 µM; and [H_2O_2] = 1 mM] and ten times more than the optimized concentration, [*denoted as 10x*; [Fe(II)] = 500 µM; [TGA] = 1000 µM; and [H_2O_2] = 10 mM] were selected. Sterilized WWTP effluent (Table S 1) was treated with 1x and 10x reagent doses for 120 mins. *E.coli* was incubated in this Fe(II)/TGA/H_2O_2 treated water (1x and 10x). For control, *E.coli* was incubated in sterilized WWTP effluent without Fenton reagents (Fe(II)/TGA/H_2O_2). *E.coli* growth was monitored for every 60 min by recording O.D at 600 nm.[11] In addition, after 6 hours of treatment, 100 µL of sample was removed, diluted, and plated. Bacterial enumeration was performed after incubation of plates at 37°C for 24 hours.

2.10 Evaluation of phyto-toxicity:

Phytotoxicity of Fe(II)/TGA/H_2O_2 treated water (1x and 10x) was tested against two model plants *Vigna radiata* and *Macrotyloma uniflorum*, by seed germination assay as described elsewhere.[12] Fifteen seeds of each plant were soaked in 20 ml of Fe(II)/TGA/H_2O_2 treated water (1x and 10x) for 12 hours. These seeds were then

transferred to petri plates covered with three pieces of whatman filter paper. These plates were irrigated with 5 ml of Fe(II)/TGA/H_2O_2 treated water. The plates were covered and incubated for 4 days at 37°C. In control, seeds were irrigated by sterile WWTP effluent. Three independent experiments in duplicates were performed, and germination index (GI) was calculated using the following formula:[12]

$$\% \text{ GI} = 100 \times (S_D/S_C) \times (L_D/L_C)$$

where S_D - number of seeds germinated for sample
S_C - number of seeds germinated for control
L_D - average root length of seeds for sample
L_C - average root length of seeds for control

3. Supporting Experiments:

3.1 Analysis of Residual Fe(II)

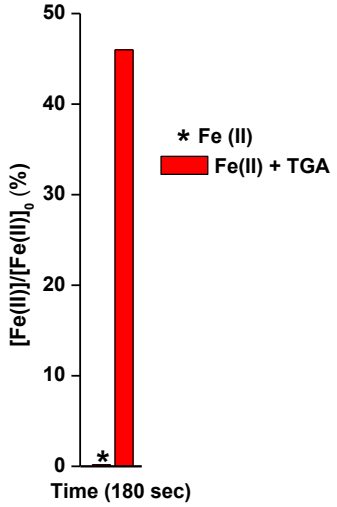


Fig. S 1 Residual Fe(II) in aqueous solution of Fe(II) in presence and absence of TGA after 3 min stirring at pH 6. [Fe(II)] = 50 µM; [TGA] = 100 µM.

3.2 Reaction between Fe(II) and TGA:

Under optimized conditions for the degradation, [Fe(II)] = 50 µM, [TGA] = 100 µM and at pH 6, the mixing of Fe(II) and TGA solution showed a flash of purple color that vanished immediately. This phenomenon is consistent with previous observations.[13] Upon increasing the reagent concentration [Fe(II)] = 1 mM, [TGA] = 2 mM and at pH 11, the solution turned to reddish purple color due to the formation of Ferro thioglycolate complex (Fig. S2A). However, upon addition of H_2O_2, the reddish purple color faded

immediately due to the oxidation of thioglycolic acid to dithioglycolate by H_2O_2 (Fig. S2A). Moreover, immediate precipitation of iron occurred because of alkaline condition. These observations were same as those reported earlier.[13] It is well know that TGA can bind with ferric and ferrous ions. The reaction between Fe(II) and TGA produces ferrothioglycolate complex that is rapidly oxidized under air saturated conditions to ferric thioglyclolate complex.[13] In presence of oxidants such as oxygen or peroxide, this complex immediately decomposes to produce Fe(II) and dithioglycolate. Fe(II) is known to catalyse the autooxidation of thioglycolic acid to dithioglycolate.

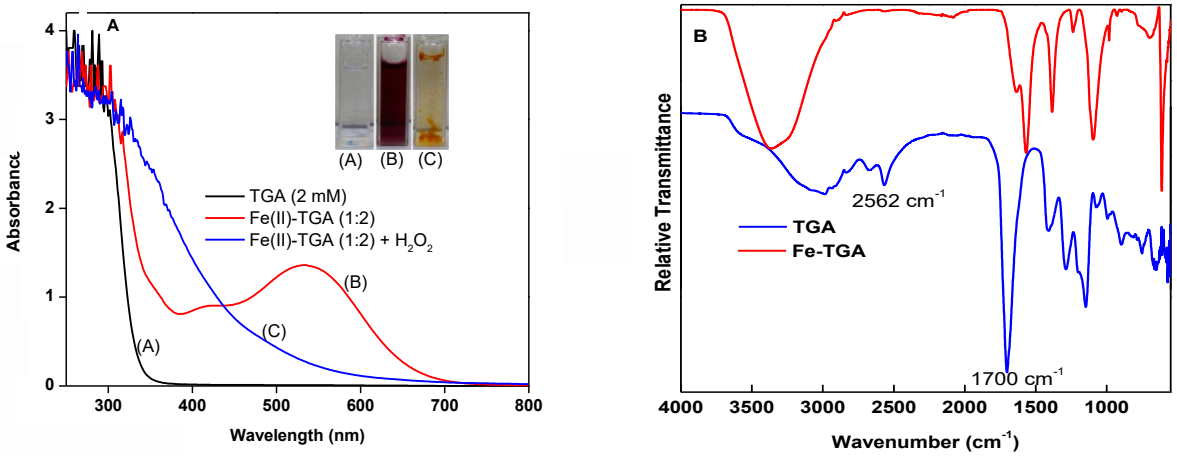


Fig. S 2 (A) Absorption spectra of TGA, TGA in presence of Fe(II) and TGA in presence of Fe(II) and H_2O_2. [Fe(II)] = 1 mM, [TGA] = 2 mM, [H_2O_2] = 10 mM, pH 11 (B) FTIR-ATR spectra of TGA and TGA in presence of Fe(II).

The complexation between [Fe(II)] = 1 mM and [TGA] = 2 mM at pH 11, was studied using Perkin-Elmer Frontier FT-NIR/MIR spectrometer under universal attenuated total reflectance (uATR-FTIR) mode. FTIR-ATR spectrum of neat TGA solution showed bands at 2562 cm^{-1} corresponding to –SH stretching vibration and at 1700 cm^{-1} due to C=O stretching vibration(Fig. S2B). On the other hand, the FTIR-ATR spectrum of TGA in presence of Fe(II), showed a dampened -SH vibration and a shift of C=O stretching vibration from 1700 cm^{-1} to 1562 cm^{-1} (Fig. S2B), revealing the complexation of Fe(II) ion with TGA.

3.3 Reusability of Fe(II) species:

Although, the degradation promoted by Fe/TGA system is a homogeneous process with Fe(II) dosage as low as 50μM, it is desirable to reuse the iron for subsequent degradation. It is important to note that after initial degradation (1st cycle), the Fe ions still remain in the solution. We examined whether these Fe ions could be

used for the subsequent degradation. Therefore, to this post treatment solution, only TGA (100 μM), MB (50 μM) and H_2O_2 (1 mM) were added (Fig. S3). Interestingly, ~83 % of MB was degraded in the 2nd cycle. Similarly, in the subsequent cycle ~63 % of MB was degraded (Fig. S3).

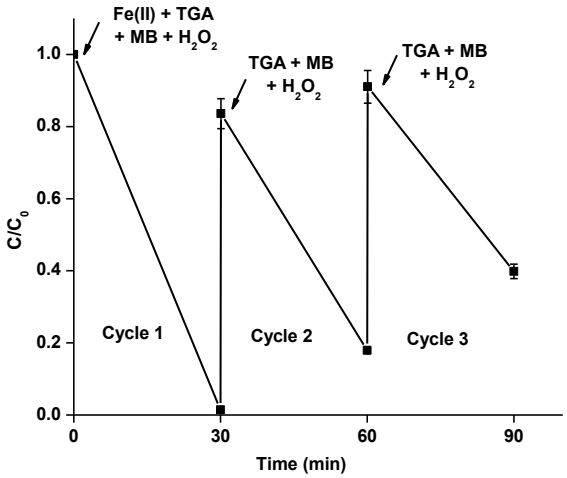


Fig. S 3 Reuse of iron species in Fe(II)/TGA/H_2O_2 system.

These results revealed that it is possible to reuse the Fe(II) ions and also reduce the net Fe(II) dosage in Fe(II)/TGA/H_2O_2 system. The results also revealed the ability of TGA to utilize Fe(II) species present in the post treatment solution for subsequent degradation. The decrease in the degradation efficiency in the 2nd and 3rd cycles is attributed to the accumulation of degraded products that compete for $^{\bullet}OH$ generated by Fe(II)/TGA/H_2O_2 system.

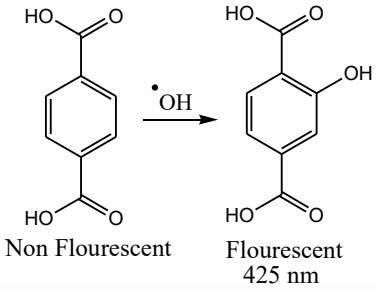
Table S 2 Kinetics of MB degradation under different experimental conditions

Condition	pH	k' (min^{-1})	$t_{1/2}$ (min)	R^2
TGA+H_2O_2	6	0.004± 0.0004	173.25	0.99
H_2O_2	6	0.001± 0.0001	693	0.89
Fe(II)+ TGA+H_2O_2 + t-BuOH	6	0.007 ± 0.001	99	0.87
Fe(II)+ Hydroxylamine +H_2O_2	6	0.002 ± 0.0013	346.5	0.98

[Fe(II)] = 50 μM; [TGA] = [Hydroxylamine] = 100 μM; MB = 50 μM; [H_2O_2] = 1 mM ; [t-BuOH] = 5 mM.

3.4 TA and NBT assays:

Terapthalic acid (TA) is a widely used chemical dosimeter for the detection of hydroxyl radicals in advanced oxidation process because TA reacts only with •OH and does not react with other reactive oxygen species such as superoxide anion radical, hydroperoxyl radical and H_2O_2. [3b, 8] TA does not show any flouresence at 425 nm. However, in presence of hydroxyl radical, TA forms a fluorescent compound, hydroxy terephthalic acid (HTA) according to the beow reaction: [3b, 8]



The fluorescence intensity of TA in Fe(II)/TGA/H_2O_2 system increased with reaction time revealing the generation of hydroxyl radicals in this system (Fig. S4 A). On the other hand TA in Fe(II)/H_2O_2 system showed negligible fluorescence revealing the absence of hydroxyl radicals (Fig. S4A). After 30 min reaction time, the •OH generated in Fe(II)/TGA/H_2O_2 system was ~38 times more than that of Fe(II)/ H_2O_2 system (Fig. S4A).

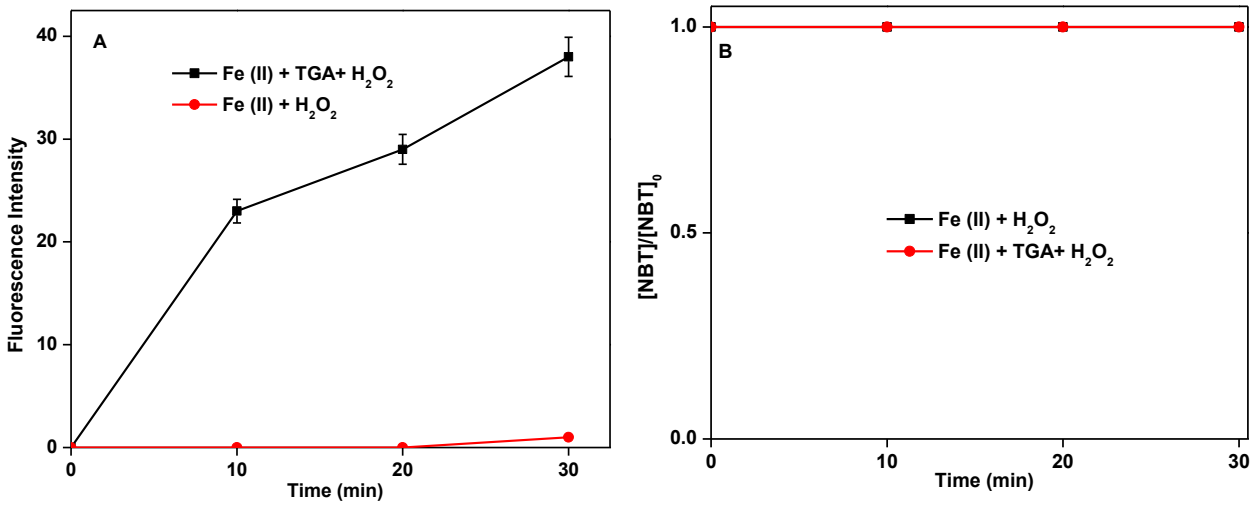


Fig. S 4 Detection of (A) OH• and (B) superoxide anion radical in Fe(II)/H_2O_2 and Fe(II)/TGA/H_2O_2 system at pH 6. [Fe(II)] = 50 µM; [TGA] = 100 µM; [TA] = 1000 µM; [NBT] = 100 µM; [H_2O_2] = 1 mM.

In addition, nitroblue tetrazolium dichloride (NBT) was employed as a probe compound to detect whether superoxide anion is formed in Fe(II)/TGA/H_2O_2 system. However, NBT assay revealed the absence of superoxide anion radical both in Fe(II)/TGA/H_2O_2 and Fe(II)/H_2O_2 system (Fig. S4B). These results imply that $^{\bullet}OH$ is the key oxidant generated in Fe(II)/TGA/H_2O_2 system.

3.5 Consumption of H_2O_2:

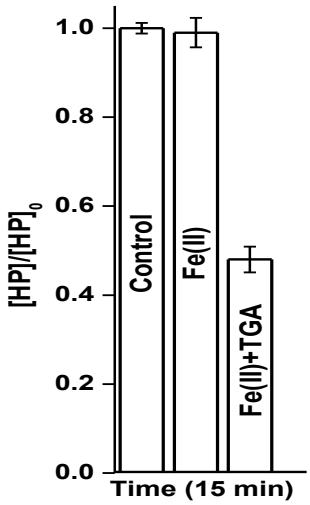


Fig. S 5 Consumption of H_2O_2 by Fe(II)/H_2O_2 and Fe(II)/TGA/H_2O_2 system at pH 6. [Fe(II)] = 50 μM; [TGA] = 100 μM; [H_2O_2] = 1 mM.

3.6 TOC analysis:

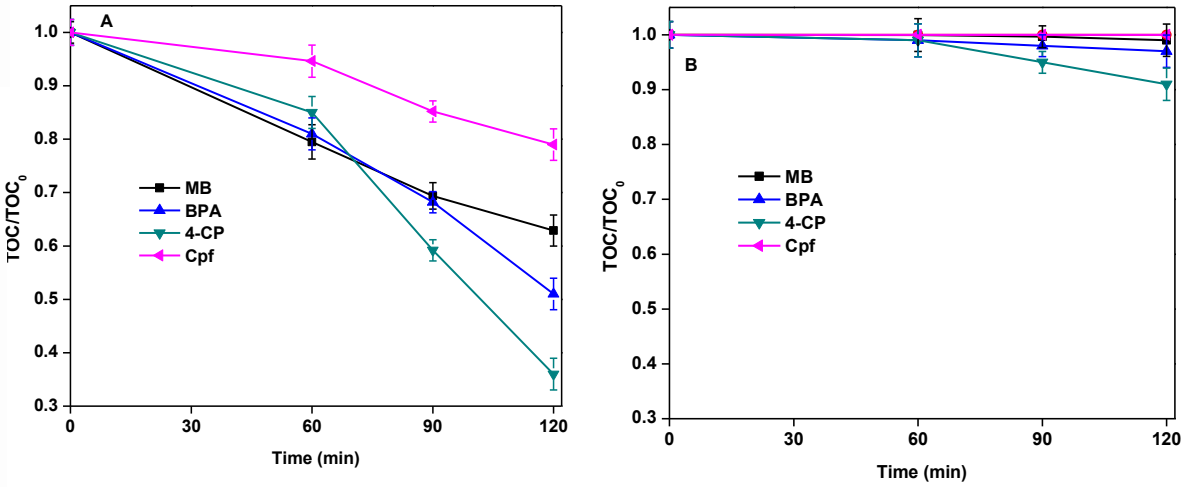


Fig. S 6 Reduction in TOC by (A) Fe(II)/TGA/H_2O_2 and (B) Fe(II)/H_2O_2 system at pH 6. [Fe(II)] = 50 μM; [contaminants] = 50 μM; [TGA] = 100 μM; [H_2O_2] = 1 mM.

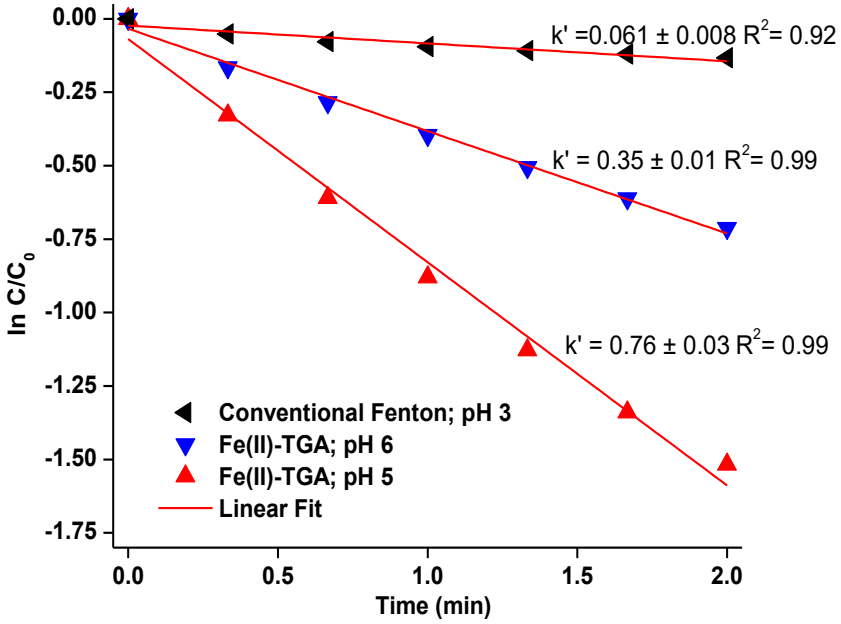


Fig. S 7 Comparison of degradation kinetics of MB by Fe(II)/TGA/H_2O_2 system at near neutral pH (5 & 6) against conventional Fenton (Fe(II)/H_2O_2) at pH 3. [Fe(II)] = 50 µM; [TGA] = 100 µM; [H_2O_2] = 1 mM.

3.7 Evaluation in simulated ground water and real water samples:

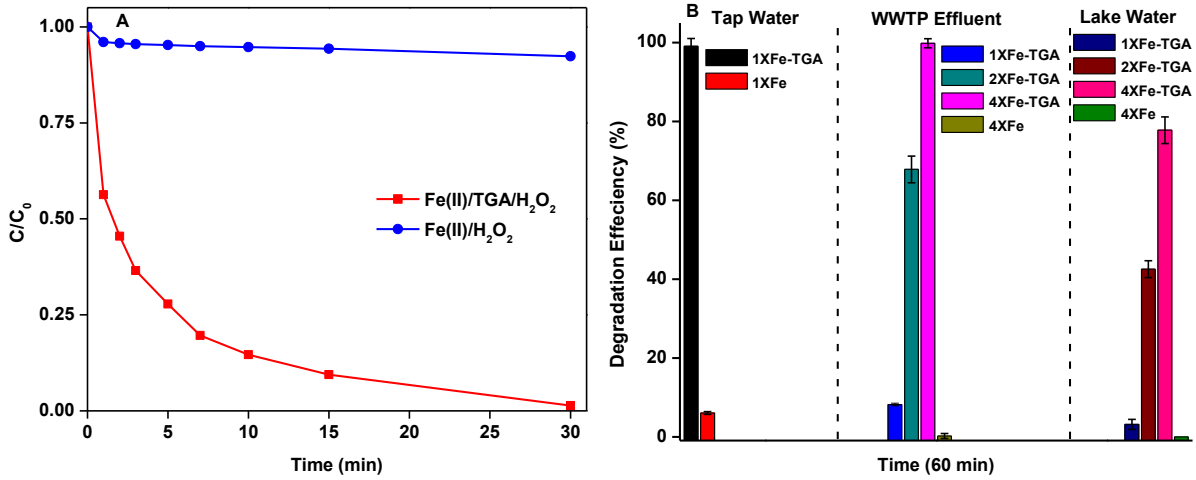


Fig. S 8 Degradation of MB by Fe(II)/H_2O_2 and Fe(II)/TGA/H_2O_2 system in (A) simulated ground water and (B) in real water samples (details section 2.6).

In case of simulated ground water and tap water, Fe(II)/TGA/H_2O_2 and Fe(II)/H_2O_2 system showed ~100% and ~5% degradation efficiency, respectively (Fig. S8). In case of relatively complex waste water matrices such as WWTP effluent and

lake water, the degradation efficiency of Fe(II)/TGA/H_2O_2 system decreased to ~8% and ~3%, respectively (Fig. S8B). However, on increasing the reagent dose to four times, the degradation efficiency increased to ~100% and ~77% in WWTP effluent and lake water, respectively (Fig. S8B). On the other hand, degradation efficiency of Fe(II) /H_2O_2 was negligible in WWTP effluent and lake water even at four times higher dosage (Fig. S8B).

It is important to note that •OH is a very strong, as well as non-specific oxidant. The significant amount of organic and inorganic constituents of WWTP effluent and lake water compete with the target contaminant for •OH thereby decreasing the degradation efficiency. However, on increasing the Fe(II)/TGA/H_2O_2 reagent dose the effective concentration of •OH required to degrade target contaminants also increase, leading to a maximum degradation efficiency. The performance of Fe(II)/TGA/H_2O_2 in real water samples for degradation followed the following order: Tap water > WWTP effluent > Lake water. Though WWTP effluent and lake water had similar organic content (COD, BOD and TOC, Table S1), the degradation efficiency in WWTP effluent was higher than lake water probably due to the absence of total suspended solids in WWTP effluent.

Importantly, these results revealed ability of Fe(II)/TGA/H_2O_2 system degrade pollutants in complex water matrices and support the applicability of Fe(II)/TGA/H_2O_2 system for real waste water treatment applications. The results also indicate the nature and the complexity of the water matrix influence the degradation efficiency. Therefore, prior to real application, it is recommended to optimize Fenton reagent dosage to achieve maximum degradation efficiency.

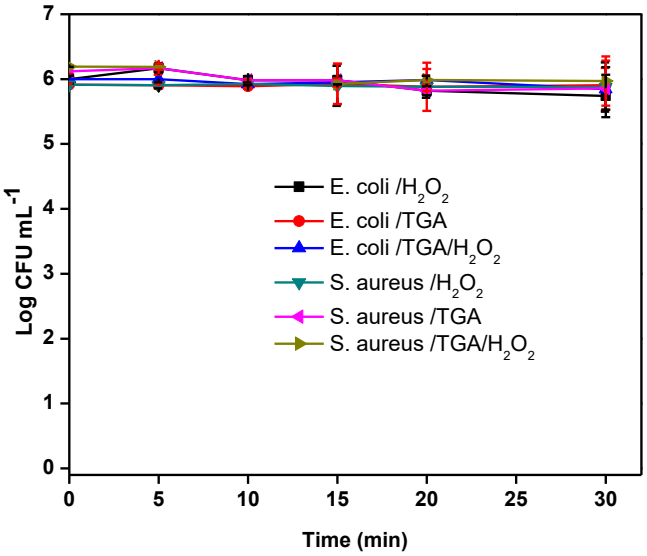


Fig. S 9 Cell viability of *E. coli* and *S. aureus* on treatment with H_2O_2 alone, TGA alone and TGA and H_2O_2 at pH 6. [TGA] = [H_2O_2] = 1 mM; [*E. coli*] = [*S.* aureus] = ~10^6 CFU ml^{-1}.

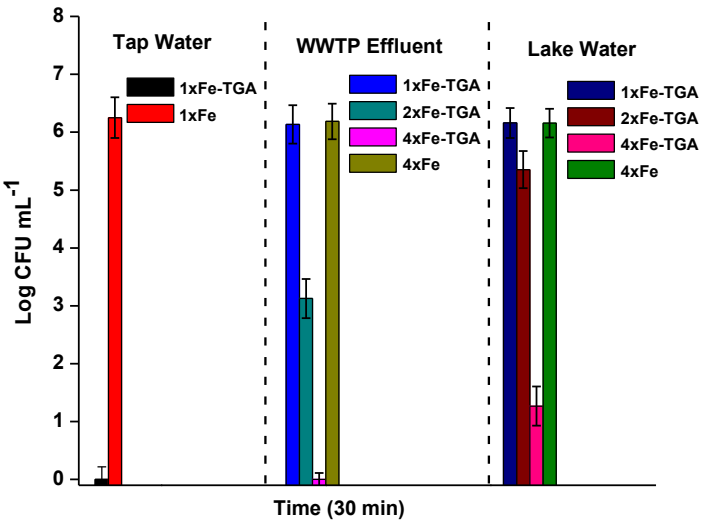


Fig. S 10 Inactivation of *E.coli* by Fe(II)/H_2O_2 and Fe(II)/TGA/H_2O_2 system in real water samples (details section 2.6).

Note: The inactivation of *E.coli* in real water samples by Fe(II)/H_2O_2 and Fe(II)/TGA/H_2O_2 showed a similar trend as observed for MB degradation (Fig. S8B). The performance of Fe(II)/TGA/H_2O_2 in real water samples for inactivation of *E.coli* followed the following order: Tap water > WWTP effluent > Lake water.

3.8 Fate and Toxicity of TGA in Fe(II)/TGA/H_2O_2 system

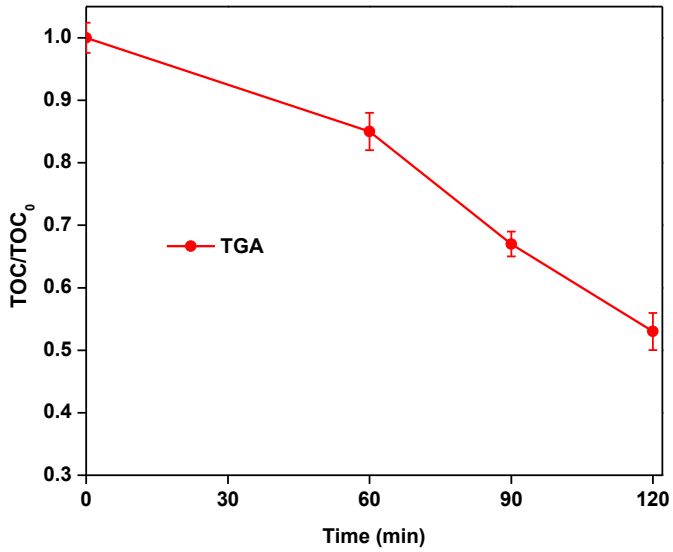


Fig. S 11 Reduction in TOC in Fe(II)/TGA/H_2O_2 system (in the absence of organic contaminants) at pH 6. [Fe(II)] = 50 µM; [TGA] = 100 µM; [H_2O_2] = 1 mM.

The fate of TGA in Fe(II)/TGA/H_2O_2 system was studied using Electro-Spray Ionization Mass Spectrometry. Mass spectrum of Fe(II)/TGA/H_2O_2 showed two major peaks with m/Z values of 64 and 184 (Fig. S 12) that could correspond to performic acid and dithioglycolate, respectively. Notably, dithioglycolate was also observed in the negative ion mode (m/Z = 181). These results reveal that a low molecular mass compound with m/Z = 64 (probably performic acid) and dithioglycolate as the main intermediates of Fe(II)/TGA/H_2O_2 system alone in absence of organic contaminant. It is known that dithioglycolate is one of the products formed on reaction TGA with Fe(III) (Reaction 3-4).[13b, 13d, 13e] The presence of dithioglycolate in the treated solution further supports reaction 4.

$$Fe(III) + HORSH \rightleftharpoons [Fe(III)(ORS)]^{+} + 2H^{+} \quad (3)$$

$$[Fe(III)(ORS)]^{+} \longrightarrow 2Fe(II) + HORS\text{-}SROH \quad (4)$$

HORSH = Thioglycolic acid

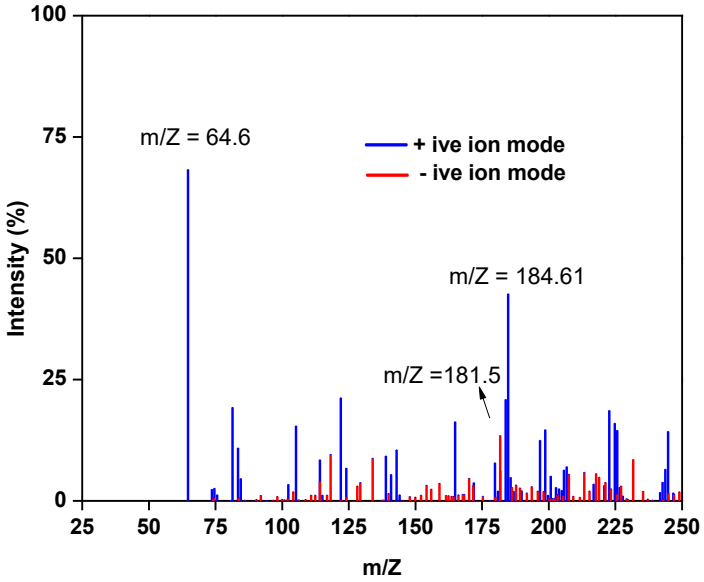


Fig. S12 ESI-MS spectra of TGA in presence of Fe(II) and H_2O_2 system.

The toxicity and phytotoxicity of Fe(II)/TGA/H_2O_2 treated water was evaluated against surrogate bacterium *E.coli*, and two model plants *Vigna radiata* and *Macrotyloma uniflorum*, respectively.

E.coli growth curves (Fig. S 13) revealed that Fe(II)/TGA/H_2O_2 treated waste water had no effect on *E.coli* growth and metabolism even at ten times higher reagent dose than the optimized dosage. Similarly, Fe(II)/TGA/H_2O_2 treated waste water showed no phytotoxic effects against both *Vigna radiata* and *Macrotyloma uniflorum* as revealed by seed germination assays (Fig. S 14 A and B). These results demonstrate that

Fe(II)/TGA/H_2O_2 system does not produce any toxic byproducts and therefore is environmentally benign.

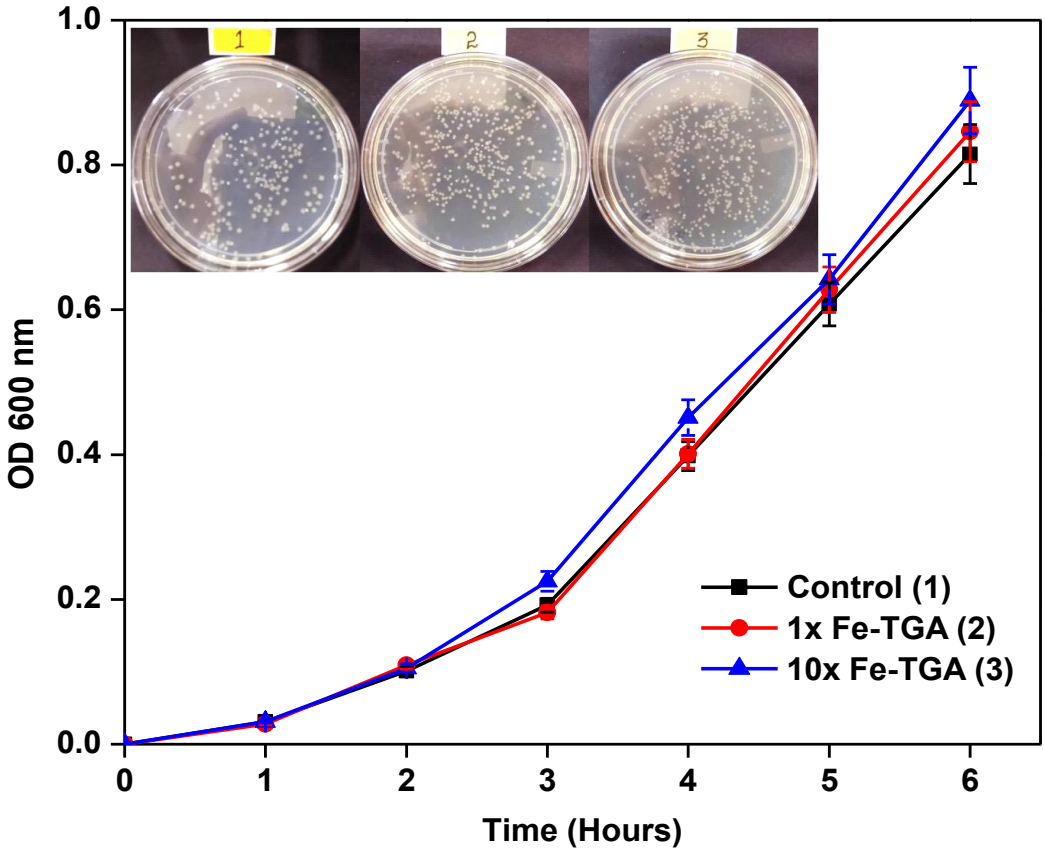


Fig. S 13 Growth kinetics of *E.coli* in presence and absence (control) of Fe(II)/TGA/H_2O_2 treated water (1x and 10x) (details section 2.9). **Inset**: Photograph showing the *E.coli* colonies after 6 hr treatment in presence and absence (control) of Fe(II)/TGA/H_2O_2 treated water (1x and 10x).

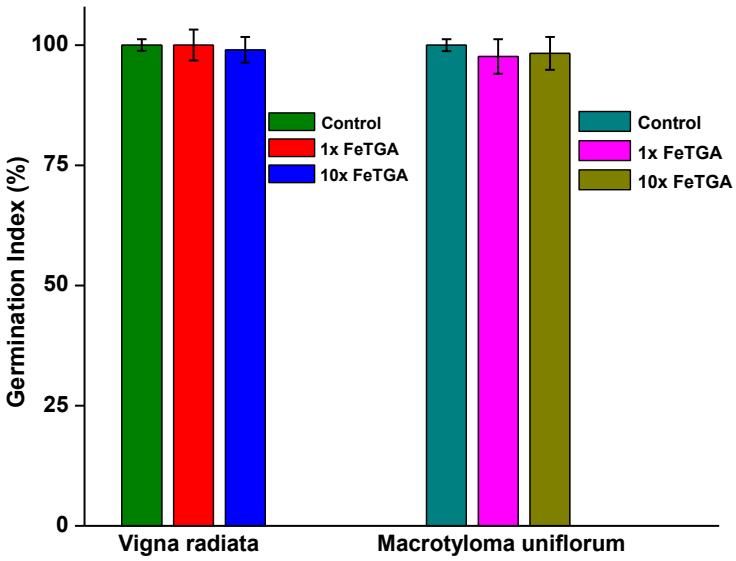


Fig. S 14 (A) Effect of Fe(II)/TGA/H_2O_2 treated water (1x and 10x) on seed germination of *Vigna radiata* and *Macrotyloma uniflorum* in comparison with control (details section 2.10).

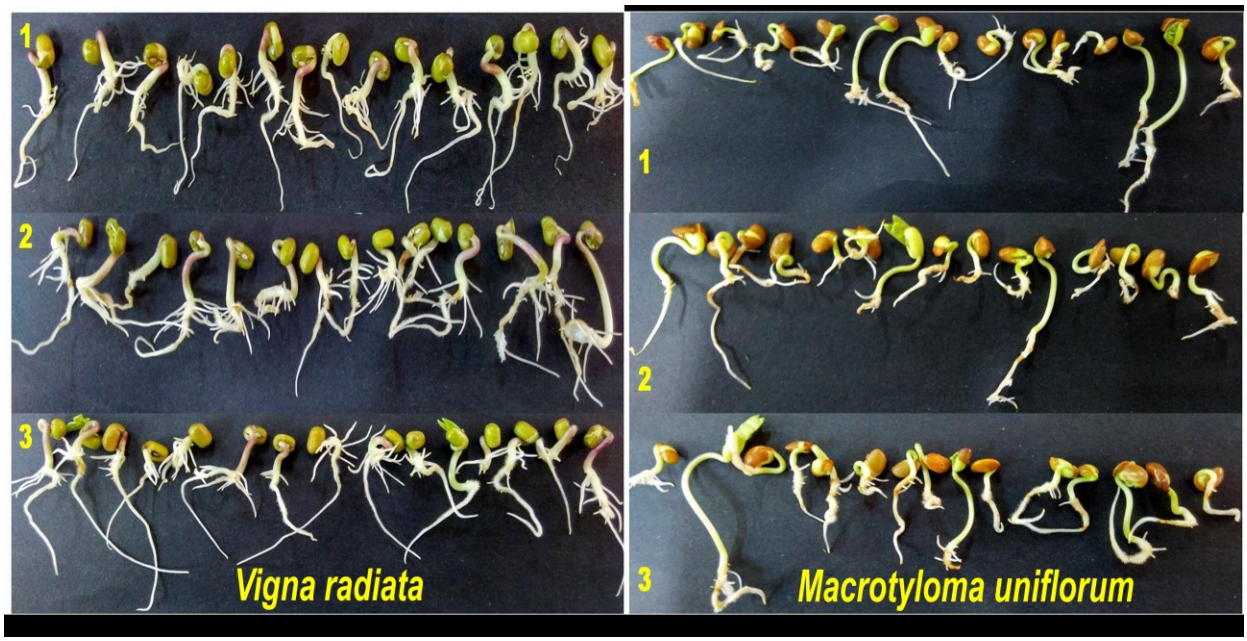


Fig. S 14 (B) Photograph showing the germination of *Vigna radiata* and *Macrotyloma uniflorum* irrigated with Fe(II)/TGA/H_2O_2 treated water (1x and 10x) in comparison with control. 1 = Control (sterile WWTP effluent in absence of Fenton reagent); 2 = Fe(II)/TGA/H_2O_2 treated water at 1x concentration; 3 = Fe(II)/TGA/H_2O_2 treated water at 10x concentration (details section 2.10).

4. References:

1 P. Y. Chan, M. Gamal El-Din and J. R. Bolton, *Water Res.*, 2012, **46**, 5672.

2 M. S. Morgan, P. F. Van Trieste, S. M. Garlick, M. J. Mahon and A. L. Smith, *Anal. Chim. Acta*, 1988, **215**, 325.

3 (a) H. Liu, T. A. Bruton, W. Li, J. V. Buren, C. Prasse, F. M. Doyle and D. L. Sedlak, *Environ. Sci. Technol.* , 2016, **50**, 890; (b) G. Subramanian and G. Madras, *Water Research*, 2016, **104**, 168.

4 R. F. P. Nogueira, M. C. Oliveira and W. C. Paterlini, *Talanta*, 2005, **66**, 86.

5 H. Fadrus and J. Maly, *Analyst*, 1975, **100**, 549.

6 W. Huang, M. Brigante, F. Wu, C. Mousty, K. Hanna and G. Mailhot, *Environmental science & technology*, 2013, **47**, 1952.

7 (a) L. Chen, J. Ma, X. Li, J. Zhang, J. Fang, Y. Guan and P. Xie, *Environmental science & technology*, 2011, **45**, 3925; (b) A. A. Vitale, E. A. Bernatene, M. n. G. Vitale and A. B. Pomilio, *The Journal of Physical Chemistry A*, 2016, **120**, 5435.

8 (a) M. Sahni and B. R. Locke, *Industrial & engineering chemistry research*, 2006, **45**, 5819; (b) J. G. Radich and P. V. Kamat, *ACS nano*, 2013, **7**, 5546.

9 C. Zhao, L. E. Arroyo-Mora, A. P. DeCaprio, V. K. Sharma, D. D. Dionysiou and K. E. O'Shea, *water research*, 2014, **67**, 144.

10 D. Eatson, S. Clesceri, E. Rice and A. Greenberg, *Centennial Edition, USA*, 2005, 4.

11 G. Sezonov, D. Joseleau-Petit and R. D'Ari, *Journal of bacteriology*, 2007, **189**, 8746.

12 A. Ginos, T. Manios and D. Mantzavinos, *Journal of Hazardous Materials*, 2006, **133**, 135.

13 (a) E. Lyons, *Journal of the American Chemical Society*, 1927, **49**, 1916; (b) D. Leussing and I. Kolthoff, *Journal of the American Chemical Society*, 1953, **75**, 3904; (c) D. L. Leussing and L. Newman, *Journal of the American Chemical Society*, 1956, **78**, 552; (d) D. Leussing and T. Tischer, *Advances in Chemistry Series*, 1963, **37**, 216; (e) K. J. Ellis, A. G. Lappin and A. McAuley, *Journal of the Chemical Society, Dalton Transactions*, 1975, 1930; (f) E. Baumgartner, M. A. Blesa and A. J. Maroto, *Journal of the Chemical Society, Dalton Transactions*, 1982, 1649.